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Effect of high day and night temperature regimes on tomato (*Solanum lycopersicum*) genotypes

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ABSTRACT

Five tomato (*Solanum lycopersicum* L.) genotypes, including heat tolerant (Pusa Sadabahar, Booster, Pusa Sheetal), one F_1 combination (Pusa Sheetal × Pusa Sadabahar) and one susceptible genotype (Pusa Rohini) were grown under phytotron under four temperature regimes, i.e. 20/24, 22/26, 24/32, 27/37°C night (11 hours)/day (13 hours) temperature, respectively. Pusa Sadabahar and Booster recorded high value of relative water content (RWC) and low value of membrane injury index (MII) both at normal and high temperature conditions. High value of chlorophyll a and chlorophyll b ratio was recorded in Pusa Sadabahar and Pusa Sheetal×Pusa Sadabahar under all the temperature regimes showed their tolerance to high temperature. Normal (more than 80 percent) flowering and fruiting was recorded in all the genotypes at 20/24°C & 22/26°C. However at 24/32°C Pusa Sadabahar recorded 65 per cent fruit set and other genotypes 25 to 49 percent fruit setting only. None of the genotypes could record fruit set at 27/37°C, except Pusa Sadabahar which could set few small fruits (19%). Pollen germination was maximum (ranging from 21.8 - 62.9%) in Pusa Sadabahar under all temperature regimes. The susceptible genotype, Pusa Rohini recorded exerted stigma in 100% flowers at 27/37°C temperature whereas it was 75% in tolerant genotype Pusa Sadabahar. Night/day temperature 22/26°C was optimum for fruit set, pollen viability and normal stigma development in tomato. High night temperature (≥ 26°C ± 2°C) at flowering was the major factor in reducing fruit set in tomato than the day temperature. The study showed that day temperature of ≥ 35°C and night temperature of ≥ 26°C may be used for screening tomato against high temperature tolerance.

Key words : Chlorophyll content, Fruit set, Membrane injury index (MII), Pollen germination, Relative water content (RWC), *Solanum lycopersicum*

Tomato (*Solanum lycopersicum* L.) is an important vegetable crop all over the world. India accounts 11% of total world's tomato production of 151 million tonnes from 0.87 million hectare area (NHB 2011). In tropical and subtropical regions, heat stress may become a major limiting factor for the growth, reproduction and yield of crop (Hall 2001). Since tomato gives high return in per unit area and per unit time therefore, it is grown almost every part of India. The vegetative and reproductive processes in tomatoes are strongly modified by temperature alone or in conjunction with other environmental factors (Foolad 2005). Tomato requires optimum temperatures for cultivation between 25° and 30°C during the photoperiod and 20°C during the dark period (Camejo *et al.* 2005). However, a 2-4°C increase over the optimal temperature adversely affects gamete development and inhibits the ability of pollinated flowers to develop into seeded fruits and thus reduced crop yield (Sato *et al.* 2001, Firon *et al*. 2006). At high temperature, most tomato cultivars have problems with fruit set, pollen meiosis and germination, ovule development and viability and development of the embryo (Peet *et al*. 1988). Seed germination, seedling and vegetative growth, flowering, fruit set, and fruit ripening are adversely affected at a temperature of above 35°C (Thomas and Prasad 2003, Wahid *et al.* 2007). The reproductive stage in tomato is more sensitive to high temperatures than the vegetative ones. These problems can be minimized by the improvement of cultural practices and breeding approaches. Selection of crops for tolerance to high temperature stress is proposed as the best and easiest strategy for breeding (Warner and Erwin 2005). The present study was carried out to evaluate the effect of high day and night temperature on vegetative and reproductive behaviour of tolerant and susceptible genotypes and develop screening criteria for high temperature tolerance.

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MATERIALS AND METHODS

The present experiment was carried out with five tomato

genotypes under control condition (phytotron) during summer 2011. The genotypes including heat tolerant namely; Pusa Sadabahar (PS), Booster (BS), Pusa Sheetal (PSH), one F_1 combination (PSH×PS) and one susceptible genotype Pusa Rohini (PR) were grown under phytotron under four temperature regimes, i.e. 20/24, 22/26, 24/32, 27/37°C night/day temperature for 11 /13 hours, respectively to evaluate the effect of high day and night temperature on vegetative and reproductive behaviour of tolerant and susceptible genotypes. Seeds of five genotypes were sown in nursery plug tray in mixture of vermiculite, peat and sand (1:1:1 ratio). After 22 days seedlings were transplanted in pots of 6 inches size having same medium and kept in Phytotron (glass chamber) under control condition (20/24°C, night/day temperature). Before flowering (after 40 days of sowing) plants were shifted in different glass chambers leaving 5 plants of each genotypes in each environmental condition at 5 days intervals in a manner that all the plants reached to different environmental conditions before flowering (60 days after sowing). The temperature of 27/37°C chamber was initially kept at 27/32°C and day temperature was slowly increased upto 37°C in 5 days @ 1°C/day (60 days after sowing reached to 27/37°C) keeping night temperature constant at 27°C to avoid sudden heat shock to plants. Tomato plants were watered regularly. First flowering was recorded after 60 days of sowing and flower clusters between 2nd to 6th flowers on each plant were used for various flower related studies. The data on vegetative and reproductive characters were recorded on regular intervals from five plants in each treatment. Relative water content (RWC) (%) was measured as suggested by Brass and Weatherley (1962) and Membrane injury index (MII) as method suggested by Blum and Ebercon (1981). *In vitro* pollen germination was measured as per method suggested by Brewbaker and Kwack (1963). Percentage of fruit set per plant was determined as the total number of fruits divided by the total flower number on flower clusters 2-6 of plant.

The method suggested by Blum and Ebercon (1981) was employed for the estimation of membrane injury index of leaf. After 7 days of keeping plant in different chambers. Accurately weighed 0.1g of freshly sampled leaf material was immersed in a test tube containing 10 ml of double distilled water. The tube was incubated at 45°C for 30 minutes in a hot water bath. Thereafter, electrical conductivity of the incubated solution (C_1) was measured with the help of a conductivity meter (Systronics India Ltd, Mumbai, India). These tubes were then incubated in hot water bath (100°C) for a period of 10 minutes. The incubated solution was cooled down to the room temperature and electrical conductivity (C_2) was measured. The membrane injury index of leaf was calculated according to the following formula: MII = C1/C2 where, MII = Membrane injury index, C1 = EC at 45°C for 30 minutes and C2 = EC at 100°C for 10 minutes

The method for determining relative water content was suggested by Brass and Weatherley (1962). In order to reduce chances of water loss from leaves the sample were kept in polythene bag and sealed properly. The bags were then placed in picnic thermocooler having temperature of 10-15°C and brought to lab as soon as possible. Collected leaves were immediately cleaned with distilled water and made into 8 mm discs with a cork borer. 10 such discs were selected and their individual fresh weight was measured and then floated over distilled water in closed petriplates for 4-6 hr. These discs were then surface dried by placing them in between 2 sheets of whatman no.1 filter paper. The saturated weight of these discs was recorded. These samples were then dried in a hot air oven at 70°C for 72 hr until constant weight is obtained. Finally the dry weight of samples was recorded. The relative water content was estimated using the following formula.

$$\text{RWC (\%)} = \frac{\text{Fresh weight} - \text{Oven dry weight}}{\text{Turgid weight} - \text{Oven dry weight}} \times 100$$

Accurately weighed 100 mg of clean leaf sample was immersed in 10 ml of DMSO (AR grade) to determine chlorophyll content. The samples were incubated at 70 ^{0}C for 4 hours in incubator. Then it was diluted 5 times and sample was read on a UV-VIS spectrophotometer at 645 and 663 nm wavelengths using pure DMSO as blank. Chlorophyll a, chlorophyll b and total chlorophyll were calculated on fresh weight basis as per the following formulae:-

$$\text{Chlorophyll a (mg/g f. w.)} = \frac{(12.7 \times OD663) - (2.69 \times OD645) \times \text{volume} \times \text{dilution}}{1000 \times \text{wt. of sample}}$$

$$\text{Chlorophyll b (mg/g f. w.)} = \frac{(22.9 \times OD645) - (4.68 \times OD663) \times \text{volume} \times \text{dilution}}{1000 \times \text{wt. of sample}}$$

$$\text{Total Chlorophyll (mg/g f. w.)} = \frac{(20.7 \times OD645) - (8.02 \times OD663) \times \text{volume} \times \text{dilution}}{1000 \times \text{wt. of sample}}$$

RESULTS AND DISCUSSION

Relative water content was maximum in genotype Booster under heat stress 27/37°C, however rate of reduction in RWC was minimum in Pusa Sheetal followed by Pusa Sadabahar and PSH×PS (Fig 1). In all the genotypes MII showed increasing trend with increase in night/day temperatures (Fig 2). Up to 24/32°C temperature slight increase in MII was recorded in all the genotypes. However, at 27/37°C all the genotypes showed drastic and significant increase in MII except Pusa Sadabahar. Pusa Rohini showed very high membrane injury index at 27/37°C. Low value of MII in tomato genotypes showed their tolerance to heat stresses. Saeed *et al*. (2007) recorded high thermostability (low membrane injury) in heat tolerant tomato genotypes. Booster and Pusa Sadabahar recorded high value of RWC

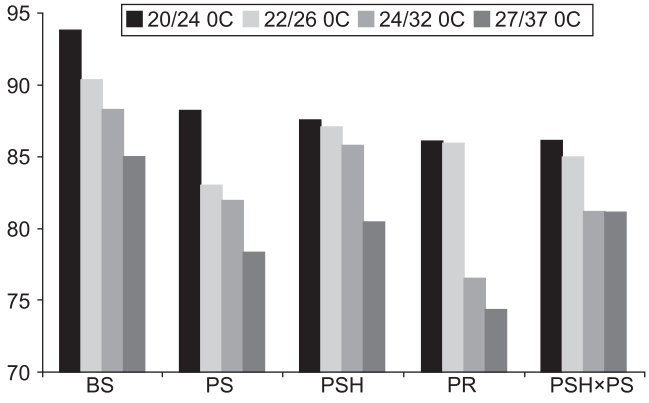

Fig 1 Relative water content under different night/day temperature regimes in heat tolerant and susceptible tomatoes.
BS=Booster, PS=Pusa Sadabahar, PSH=Pusa Sheetal, PR=Pusa Rohini.
The LSD (P≤0.05) for 20/24°C = 0.79, 22/26°C = 0.50, 24/32°C = 0.83, 27/37°C = 0.52

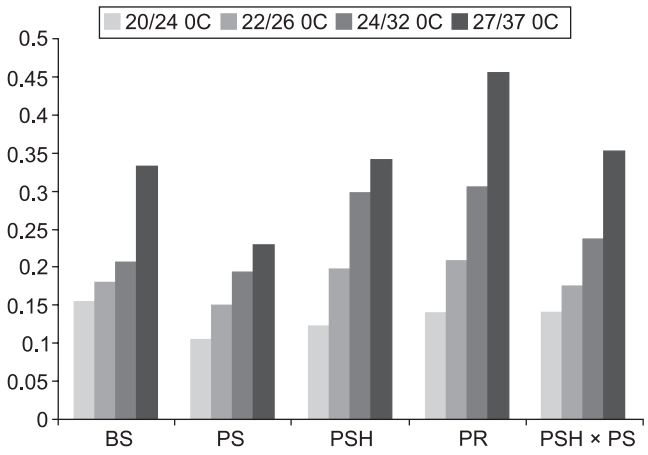

Fig 2 Membrane injury index under different night/day temperature regimes in heat tolerant and susceptible tomatoes.
BS=Booster, PS=Pusa Sadabahar, PSH=Pusa Sheetal, PR=Pusa Rohini.
The LSD (P≤0.05) for 20/24°C = 0.01, 22/26°C = 0.02, 24/32°C = 0.01, 27/37°C = 0.02

and low value of MII both at normal and high temperature condition, therefore, these genotypes has shown tolerance to heat stress. Pusa Rohini showed least tolerance to high temperature on the basis of RWC and MII value.

Chlorophyll content in different tomato genotypes under different temperature regimes showed that chlorophyll a content increased in all the genotypes with increase in temperature. However maximum increases in chlorophyll a was recorded in PSH × PS followed by Pusa Sadabahar. Similarly PSH × PS, Booster and Pusa Sadabahar also recorded high value of chlorophyll b with increasing level of temperatures. Susceptible genotype, Pusa Rohini recorded lowest value of chlorophyll a and decreasing level of chlorophyll b with increases in environmental temperature. High value of chlorophyll a/b ratio was recorded in Pusa Sadabahar and PSH×PS under all the temperature regimes showed their tolerance to high temperature, while Pusa Rohini again recorded lower value of chlorophyll a/b ratio showing its susceptibility to high temperature, thus chlorophyll a/b ratio found to be one of the reliable parameters for screening tomato genotypes against heat (high temperature).High leaf chlorophyll content was reported in tomato under sub optimal temperature stress than in the optimal period (Ntatsi *et al*. 2013).

All the genotypes showed high fruit set at 20/24°C and increase in fruit set was recorded at 22/26°C (Table 1) but at 24/32°C drastic reduction in fruit set was recorded in all the genotypes. Susceptible genotype Pusa Rohini showed 70% reduction in fruit set when temperature increased from 22/26°C to 24/32°C. Therefore 24/32°C night/day temperature was found critical and beyond this drastic reduction in fruit set was noticed. The 38% and 68% reduction in flowering in tolerant and susceptible genotypes of tomato was also recorded at high temperature under field condition respectably (Aref and Baki 1991). At 27/37°C none of the genotypes could set fruit, except Pusa Sadabahar, which could set 19% fruits, however fruits were small and remained immature. It was also observed that at night temperature > 26°C and day temperature 32°C, no fruit set was recorded in almost all genotypes, except Pusa Sadabahar which could set few fruits. In contrast to this at day temperature > 32°C and night temperature 20 - 22°C fruit setting was satisfactory in all the genotypes.

The most noticeable effect of high temperature on reproductive processes in tomato was the production of an exserted style (i.e. stigma is elongated beyond the anther cone), which may prevent self-pollination and ultimately affects fertilization and post fertilization processes leading to reduced crop yield. There was no stigma elongation in any genotype when night/day temperature was 20/24°C and 22/26°C. At temperature 24/32°C stigma elongation was recorded in all the genotypes, except Pusa Sadabahar. At 27/37°C temperature 75% flower stigma elongation was recorded in Pusa Sadabahar whereas in other genotypes almost 100% stigma elongation was recorded, therefore, none of the genotype could set fruit at 27/37°C except Pusa Sadabahr, thus Pusa Sadabahr could show tolerance to high night and day temperature up to 26°C and 35°C respectively. It can be concluded that the genotypes producing flower with normal stigma tube under high temperature would produce high fruit yield.

Pollen germination studies showed that germination of pollen increased in all genotypes when night/day temperature increased from 20/24 to 22/26°C. But at 24/32°C reduction in pollen germination was recorded in all genotypes (Table 1). However at 27/37°C drastic reduction in pollen grain germination was recorded and susceptible genotype Pusa

Table1 Effect of different night/day temperature regimes on fruit set (%), stigma elongation

Genotype	Fruit set (%)				Stigma elongation (%)				Pollen germination (%)			
	20/24°C	22/26°C	24/32°C	27/37°C	20/24°C	22/26°C	24/32°C	27/37°C	20/24°C	22/26°C	24/32°C	27/37°C
Booster	80.0	85.3	45.7				30.1	95.3	32.8	37.9	35.8	7.1
Pusa Sadabahar	84.5	89.5	65.0	19.0			0.0	75.2	51.1	62.9	55.6	21.8
Pusa Sheetal	83.4	88.7	37.5				28.1	98.1	36.8	45.5	38.1	9.5
Pusa Rohini	81.7	86.2	25.0				65.2	100.0	40.6	56.2	49.7	1.0
PSH×PS	85.5	88.5	49.3				27.6	93.5	37.1	39.1	31.2	8.3
CD (P=0.05)	3.8	2.4	2.1					5.4	1.4	2.6	1.8	1.6
CV (%)	2.5	1.5	1.1					3.1	1.9	2.9	2.3	8.0

(%) and pollen germination (%) of heat tolerant and susceptible tomatoes.

Rohini recorded only 1% pollen germination whereas tolerant genotype Pusa Sadabahar recorded 21.8% pollen germination. Highly significant differences for pollen germination were recorded among different tomato genotypes. Thus, pollen germination at high temperature may be an effective selection criteria for heat tolerance.

Optimum temperature for flowering and fruiting of tomato has been reported 20°C night and 25°C during day (Camejo *et al.* 2005), however in our finding 22°C night and 26°C day temperature was found optimum for fruit set, pollen germination and normal stigma development in tomato. It was concluded that increase in temperature beyond 26°C played a major role in reducing fruit set, pollen viability and stigma receptivity in tomato rather than day temperature. Therefore, night temperature ≥ 26°C and day temperature ≥35°C may be used for screening tomato against high temperature.

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